

Evaluation of Diagnostic Techniques for Scrub Typhus

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Abstract

Background: Acute febrile illness (AFI) is one of the common medical issue in developing countries including India. The common causes of AFI are enteric fever, malaria, UTI etc. Among the uncommon ones that may be cause of AFI is scrub typhus especially in hilly areas. Untimely diagnosis & treatment may cause significant mortality & morbidity.

Aim & Objectives : 1) To determine the occurrence of scrub typhus in a tertiary health care hospital in Uttarakhand. 2) To evaluate the efficacy of diagnostic modalities for scrub typhus.

Material & Methods: The retrospective data, of over a period of 12 months was collected, of patients presenting with AFI. All the clinically diagnosed cases of AFI, were initially subjected to routine investigations for PUO (pyrexia of unknown origin) and cases which were negative were further assessed to exclude scrub typhus by W/F (Weil Felix), ICT (Immunochromatography) & IgM ELISA.

Results: Out of total 2000 cases, 380 (19%) were positive for scrub typhus. Most common age group affected was of 30-39 years. As per gender is concerned male predominance was seen in positive cases of scrub typhus. IgM ELISA was found to have sensitivity of 96.84% and specificity of 100%

Conclusion: Scrub typhus is endemic in the hilly areas such as Dehradun and has also emerged as an important cause of AFI. Amongst the various diagnostic methods, IgM ELISA has been found to have good sensitivity & specificity, though this test is costlier & a time consuming procedure.

Keywords: Orientia tsutsugamushi, acute febrile illness, scrub typhus, Weil Felix, ICT, ELISA

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I. Introduction

Fever of unknown origin (FUO)/ Pyrexia of unknown origin (PUO) is defined as the body temperature increases to 38.3°C (101°F) or more several times a day lasting longer than 3 weeks or failure to reach a diagnosis despite one week of inpatient evaluation.¹ Although there are multiple causes of FUO but infections such as enteric fever, malaria, dengue, tuberculosis, brucellosis are among most common causes.² Scrub typhus (ST), a rickettsial disease caused by *Orientia tsutsugamushi*, is a very less known cause of FUO.³ Rickettsial infections are re-emerging with increased reports from different parts of the world. Scrub typhus is an acute febrile illness and delay in diagnosis is associated with considerable morbidity and mortality. Although it is rare disease in India, but in recent years there has been reports from Maharashtra, Tamil Nadu, Karnataka, Kerala, Jammu and Kashmir, Uttaranchal, Himachal Pradesh, Rajasthan, Assam and West Bengal indicating the re emergence of the disease.^{4,5,6,7,8,9}

Scrub typhus, an acute febrile disease caused by *Orientia tsutsugamushi*, is a Gram negative, obligate intracellular bacterium. It is transmitted to humans by the bite of the larval stage of trombiculid mites (chiggers)¹⁰.

The name is derived from the occurrence of the mites in regions of scrub vegetation. The disease is endemic in the geographical region known as "tsutsugamushi triangle" which extends from northern Japan and far-eastern Russia in the north, to the territories around the Solomon Sea into northern Australia in the south, and to Pakistan and Afghanistan in the west. The bite of this mite causes a characteristic black eschar which is a differentiating feature in making the diagnosis.^{5,11} Several tests are available with their own advantages and limitations.¹² Microimmunofluorescence, latex agglutination, indirect hemagglutination, immunoperoxidase assay and enzyme linked immunosorbent assay are the tests available for the diagnosis of rickettsial infections.¹³ Immunofluorescence assay (IFA) is the "gold standard" technique and is used as a reference technique in most laboratories. Among all the serological tests available W/F is the cheapest and easily available but this is notoriously unreliable. Dot blot immunoassay is considered as alternative to the Weil Felix test, for the rapid diagnosis rickettsial infections in areas with a high occurrence.¹⁴ IgM ELISA has been evaluated and found to be satisfactory in comparison to the gold standard. The rapid colloidal gold

immunochromatographic test (ICT) is one which detects IgM and IgG antibodies against *O. tsutsugamushi* in serum samples from patients. Serological methods based on the recombinant 56 kDa protein include a passive hemagglutination test, enzyme linked immunosorbent assay (ELISA) and dipstick test¹⁵. These tests are easy to perform and are adequate for screening a large number of samples. Doxycycline is the drug of choice. Other drugs such as chloramphenicol & azithromycin also appears to be effective. The present study was designed to determine the occurrence of scrub typhus in the hilly belt of Uttarakhand and to evaluate the diagnostic modalities for the diagnosis of *O. tsutsugamush* infection.

II. Materials and Method

Study Design : Retrospective study

Study Location : This study was done in a tertiary care teaching hospital based study done in Department of Microbiology, at SRHU, Jolly Grants, Dehradun.

Study Duration : October 2016 to September 2017.

Sample size: The sample size was 2000 cases.

Subjects & selection method: The study population was drawn from OPDs from patients with a clinical diagnosis of AFI who presented to SRHU, Jolly Grants and were prescribed microbiological investigations for the clinical diagnosis of AFI.

Clinical data, including the duration of fever, associated symptoms, vital signs, and the general, and systemic examination findings, were recorded., Complete blood counts, chest X- rays, tests for renal and liver function, urinalysis and serum electrolytes estimation were performed at the time of presentation for all AFI cases and were repeated if necessary. Common infectious conditions that could clinically mimic scrub typhus were ruled out by performing the following test: peripheral blood smear and rapid antigen test for malaria, Widal test for Enteric fever, Dengue ELISA (NS1 antigen and IgM antibody) test, urine and blood cultures. Tuberculin test, leptospira serology and an HIV testing (as per NACO guidelines) were performed wherever clinically indicated. Cardiac evaluation and cerebrospinal fluid analysis were performed for selected cases with suspected myocarditis or meningoencephalitis respectively. Ultrasound abdomen and computerized tomography scan were also done whenever necessary.

Inclusion criteria :

The study group included all the patients irrespective of age and gender presenting with chief complains of fever more than 7 days and less than 14 days duration.

Exclusion criteria:

1. Pregnant women.
2. Patients with genetic disorders.
3. Patients presenting with fever more than 14 days.

Procedure methodology

The samples negative by the initial routine investigations were further processed for scrub typhus- W/F, ICT and IgM ELISA (as per kit's litratures). 5ml of blood samples were collected in EDTA vial & plasma was separated by centrifugating at 2000 rpm for 15 minutes. The obtained plasma samples were processed as follows by the tests given below as per kit's instruction-

1. Weil Felix commercially available kit (Tulip Diagnostics kit), titer of more than 80 against OXK in Weil Felix reaction were considered to be positive (as indicated by the manufacturers instruction).
2. Immunochromatography (S.D Bioline kit)
3. IgM ELISA (In BIOS International kit)

Statistical Analysis

The results obtained was analysed statistically by using SPSS 17 version.

III. Results

A total number of 2000 blood samples were received from clinically diagnosed cases of AFI who were subjected to microbiological investigations. Out of 2000 cases 1620 were found to be positive for other causes of AFI. Scrub typhus were found to be positive in 380 cases only by using various tests- Weil Felix , ICT, IgM ELISA. Thus the occurrence was found to be 19% (380/2000 X 100) in a hilly region at Dehradun. The most common age group affected was (30-39) yrs of age. The males (247) outnumbered females (133) with the ratio of 1.8:1. (Table 1)

Out of 380 cases scrub typhus positive cases 126(33.15%) were positive by Weil Felix, 101(26.57%) positive by ICT and IgM ELISA detected 368 (96.84%) cases (Table 2).

IgM ELISA could detect 368 positive cases of scrub typhus out of which 203 were positive only by ELISA & negative by the other two rapid tests. However, 34 cases were detected positive by all the three tests, 72 cases were found positive by combination of (ELISA & W/F) and negative by ICT. (Table 3)

The sensitivity of IgM ELISA were calculated, and found to be 96.84% and specificity 100%. The IgM ELISA had PPV of 100% while the NPV was 50% (Table 4).

Table 1: Age and sex wise distribution of scrub typhus cases (n=380)

AGE GROUP	MALE	FEMALE	TOTAL
18 -29	70	37	107
30 -39	92	36	128
40 -49	54	42	96
50 -59	17	06	23
>60	14	12	26
TOTAL	247	133	380

Table 2 : Positivity of different tests for scrub typhus (n=380)

NO OF PATIENTS	WEIL FELIX	ICT	IgM ELISA
107	32	34	104
128	56	44	132
96	19	14	84
23	13	05	33
26	06	04	15
380	126 (33.015%)	101 (26.57 %)	368 (96.84%)

Table 3: Distribution of Scrub typhus cases by combination of different Diagnostic test

NO. OF POSITIVE CASES							
ELISA (+) WF (+) ICT (+)	ELISA (+) WF (+) ICT (-)	ELISA (+) WF (-) ICT (+)	ELISA (+) WF (-) ICT (-)	ELISA (-) WF (+) ICT (+)	ELISA (-) WF (+) ICT (-)	ELISA (-) WF (-) ICT (+)	ELISA (-) WF (-) ICT (-)
34	72	59	203	06	04	02	00

Table 4 :Statistical analysis of IgM ELISA

Sensitivity	96.84%
Specificity	100 %
PPV	100 %
NPV	50 %

IV. Discussion

Scrub typhus is a rickettsial disease caused by *O. tsutsugamushi*, which is a Gram negative, intracellular bacterium. It is transmitted by the bite of mite belonging to the genus *Leptotrombidium* (*L. deliienis*) in India.¹⁶ The prevalence of scrub typhus varies from 0-8% to 60%, globally.¹⁷ A study done in Christian Medical College and Hospital, Ludhaina in 2014 reported that out of 772 fever patients, 12.69% positive for scrub typhus.¹⁸ This finding is consistent with our study, where 2000 patients of AFI were evaluated , 380(19%) were found positive for scrub typhus. Another study done in Nehru Hospital of Postgraduate Institute of Medical Education and Research, observed that 24% of patients with unexplained febrile illness had scrub typhus.¹⁹ A study done in tertiary health care institution in Goa found that 34% of fever cases were positive for IgM antibodies against *O.tsutsugamushi*.²⁰ Bhutia and Pradhan, in 2013, screened 204 samples, 63 patients were confirmed positive among which 42 were males and 21 were females.²¹ The study by Ramyasree et al. (2015) reported that out of 39 patients 23 were males and 16 were females.²² Study done by Mahajan (2014) reported that among scrub typhus positive samples, 24% were male and 14.6% were females.²³ In a study from Thailand, the positivity for scrub typhus was 59.5% with highest prevalence of 77.7% seen in 40-49 years age group with no difference between the two sexes.²⁴ Gurung et al. tested 204 patients with fever of unknown origin, of which 63 were confirmed positive (30%) , out of which 42 were males and 21 were females.²¹ In our set up, out of 380 ST positive cases, males(247) outnumbered females (133) with the ratio of 1.8:1, which is well correlated with other studies.^{21,22} The present study shows more positivity of ST particularly between 30-39 years of age comprising of 128 cases which are the young and active population involved in the occupational activities where visit to forests and accidental exposure to rats are frequent. Another study done by Vivekanandan M et al. in Pondicherry reported female preponderance in their study.²⁵

Most of the symptoms of scrub typhus are non specific in nature, including fever, breathlessness, nausea, headache, vomiting and myalgia. Fever with chills and rigors (100%) was the most common presentation in our study ,which is similar to a hospital-based study done by Dass et al., from the state of Meghalaya, India.²⁶ Fever, cough and vomiting have been reported as major complaints in other studies.^{26,27} The most common complication noticed was hepatitis(80%) followed by acute respiratory distress syndrome (60%) and acute renal failure (33%) in the study of Kedareshwar et al. (2012).²⁰ Tsay and Chang (1998) reported serious complications like pneumonitis (36%) acute respiratory distress syndrome (15%), acute renal failure (9%), myocarditis (3%) and septic shock (3%).²⁸ However adverse complications were not observed in any of the cases in our study.

A high index of suspicion is required to diagnose rickettsial infections especially in endemic areas. Due to lack of simple diagnostic tools the diagnosis of scrub typhus becomes difficult. For diagnosis of ST, gold standard tests are immunofluorescence antibody (IFA) and immunoperoxidase (IIP) tests which requires highly trained personnel for interpretation of results and is expensive and not easily available in India.¹⁰

Therefore, we compared the rapid method with IgM ELISA for the diagnosis of scrub typhus. In the present study 368(96.84%) patients were found positive by IgM ELISA while Weil Felix detected 126 (33.01%) positive cases and ICT diagnosed 101(26.57%) positives. Similar study done by Kammili et al., from Secunderabad, India reported out of 100 provisionally diagnosed cases of dengue fever, 19 were found to be positive for antibodies for scrub typhus by rapid immunochromatography and Weil-Felix test.²⁹

A study conducted by Mittal et al. in Delhi on fever of unknown origin showed that 42.6% of the patients sera were positive for OXK, by Weil Felix.⁹ In another study conducted by kamarasu et al . in the state of Tamil Nadu showed 9.2% of patients sera were positive for scrub typhus and 4.6% for other rickettsial infections.⁸ In our study more scrub typhus cases were detected positive by ELISA as compared to Weil Felix and ICT, similarly Gurung et al. could also detect more scrub typhus cases by ELISA and ICT tests than Weil-Felix test.²¹ Isaac et al. have demonstrated that Weil Felix had very low sensitivity of 30% but the specificity and positive predictive value were 100%.³⁰ In another study by Prakash et al., in 2006, Weil-Felix test showed sensitivity and specificity of 43% and 98% respectively.³¹ La Scola et al. could find good correlation between the results of Weil-Felix test and detection of IgM antibodies by IFA/ELISA.¹⁴

Chinprasatsak et al.,³² evaluated a rapid dot blot assay for diagnosis of scrub typhus and reported the sensitivity and specificity of 87% and 94%, respectively in febrile patients done in 2003. They also reported that the dot blot immunoassay dipstick test was accurate, rapid, easy to use and relatively inexpensive. Jang et al. reported sensitivity of 96.3% for IgG IFA and of 100% for IgM IFA. They evaluated the specificity of IgM capture ELISA of 99%.³³ In the present study IgM ELISA reported specificity of 100% which is concordant with Jang et al.³³ In other study by Liuan Z et al. could also detect the sensitivity of combination test of IgM and IgG was 100%.³⁴ In other study from San Francisco in 1980 the sensitivity of IgM ELISA was 98.2% and specificity was 94.4%.³⁵ In our study comparing IgM ELISA over other tests showed statistically significant values of sensitivity 98.84% and specificity 100% , indicating the superiority of ELISA and justifying our findings. On the basis of our analysis the scrub typhus IgM ELISA appears to provide accurate results for the diagnosis of acute scrub typhus in a tropical setting where scrub typhus is endemic. However the total antibody testing is not diagnostically helpful in endemic areas because many patients are likely to have past scrub typhus infection and thus have the resulting IgG antibodies .

PCR is a useful and convenient alternative in the diagnosis of scrub typhus. However PCR can detect positive cases even in treated patients of pyreia of unknown origin.³⁶ Kim DM et al. in 2010 reported that Weil-Felix is a commonly used inexpensive serological test which lacks both sensitivity and specificity,³⁷ which is well correlated with our present study. The use of the Weil-Felix should be reserved only for situations where other serological tests are not available.¹³ ELISA is an easy and comparatively economic test but the results of this test may not be available on the same day as samples need to be pooled for testing thus causing delayed diagnosis and treatment.

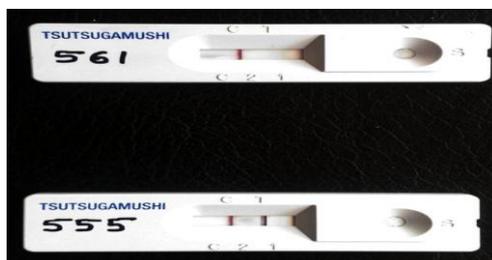
V. Conclusion

Scrub typhus is endemic in hilly belts of India and therefore surveillance becomes mandatory. Scrub typhus has emerged as an important cause of acute febrile illness, therefore, it should be considered in the differential diagnosis of patients suffering from AFI. Among the different diagnostic techniques for scrub typhus ELISA based system is helpful for detection of IgM antibodies with good sensitivity & specificity. On the other hand, rapid tests such as W/F and ICT proved to be promising screening tests, cost effective and can be performed in small laboratories.

LIMITATION

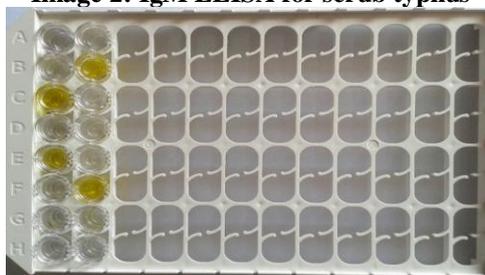
1. Due to limited resources we could not perform indirect immunoperoxidase & indirect immunofluorescence antibody assay which are considered to be gold standard tests for Scrub typhus.
2. Utilization of molecular methods would have helped in the diagnosis of missed cases of scrub typhus.
3. Results would have been more promising if the study would have carried out for longer duration of time

Image 1: Immunochromatographic test for Scrub typhus



Positive & negative tests by ICT strip for Scrub typhus

Image 2: IgM ELISA for scrub typhus



IgM ELISA microtiter plate where 1A & 1B is the positive and negative controls, wells 1C, 1E, 2B & 2F are positive.

Image 3: Qualitative slide Weil-Felix agglutination test



Weil felix slide agglutination test, wells A, C & D are positive while wells B, E & F are negative.

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